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Distribution of HCV Genotypes Among Blood Donors, Patients With Chronic Liver Disease, Hepatocellular Carcinoma, and Patients on Maintenance Hemodialysis in Korea

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Hepatitis C virus (HCV) is a single-stranded RNA virus related to the Flaviviridae family, and striking nucleotide sequence diversity has been reported among HCV isolates from different geographic areas. To study the distribution HCV genotypes among disease groups in Korea, we subtyped HCV using the method of Okamoto et al. [(1992a): *Journal of General Virology* 73:673–679] and the reverse hybridization method (INNO-LiPA) on 138 patients who were HCV polymerase chain reaction (PCR)-positive: 30 blood donors, 30 with hepatocellular carcinoma (HCC), 33 with chronic hepatitis, 15 with liver cirrhosis, and 30 patients on maintenance hemodialysis in Korea. In 30 blood donors, HCV genotype 1b was most dominant (80%), followed by genotype 2a (13.3%), and 2b (6.7%). In 30 HCC cases, HCV genotype 1b was less frequent (60%), compared to blood donors, followed by genotype 2a (33.3%), and unclassified (6.7%). In 33 chronic hepatitis cases, HCV genotype 1b was also dominant (63.6%), followed by genotype 2a (30.3%), and 1a (6.1%). In 15 patients with liver cirrhosis, HCV genotype 1b was also dominant (60%), followed by genotype 2a (33.3%), and 1a (6.7%). In 30 patients on maintenance hemodialysis, HCV genotype 1b was dominant (86.7%), followed by genotype 2a (13.3%). In conclusion, among 138 HCV PCR-positive patients, type 1b was the prevailing type (71%), followed by type 2a (23.9%), type 1a (2.1%), type 2b (1.5%), and unclassified (1.5%) in Korea. The prevalence of type 1b in blood donors (80%) was higher than in patients with liver disease (61.5%) and the prevalence of type 1b was the lowest in patients with HCC (60%).

KEY WORDS: HCV, genotype, distribution, Korea

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of post-transfusion non-A, non-B hepatitis [Alter et al., 1989]. Molecular cloning of HCV revealed that it is a single-stranded RNA virus related to the Flavivirus family [Choo et al., 1989]. Most RNA viruses exhibit extensive genetic diversity of the RNA genome. A striking diversity of nucleotide sequences has been reported among HCV isolates from different geographic areas [Cha et al., 1993]. Thus, the quasispecies nature of HCV appears to be crucial for understanding the long-term, chronic persistent infection of HCV [Martell et al., 1992; Weiner et al., 1992] and is therefore being intensively investigated. The heterogeneity of the viral genome changes cell-specific tropism [O'Brien et al., 1990; Shioda et al., 1991; Westervelt et al., 1991], the replication kinetics of viruses [Feriyo et al., 1988], cytopathic activities [Tersmette et al., 1989], the response to neutralizing antibodies [Albert et al., 1990; Nara et al., 1990], and the response to cytotoxic T lymphocytes [Messing et al., 1983]. HCV is associated with high incidence of hepatocellular carcinoma (HCC) in Japan [Saito et al., 1990]. The worldwide incidence of HCV infection averages 1.0%, depending on the area, varing from 0.5% to 1.5%. While the incidence rate of HCV infection is similar in Japan and the U.S.A., the incidence rate of HCC associated with HCV infection differs markedly [Resnick and Koff, 1993]. The heterogeneity of the HCV as well as many environmental factors may contribute to this difference.

There are six major HCV genotypes and at present at least 12 subtypes are reported [Bukh et al., 1993], although classification of these subtypes is not yet standardized. The classification of Okamoto et al. [1992a, 1993] and that of Simmonds et al. [1993a] are widely used (Table I). HCV genotype distribution in Korea has

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TABLE I. Proposed Classifications of HCV Genotypes Nomenclature

Simmonds[a]	1a	1b	1c	2a	2b	2c	3a	3b	4a	5a	6a
Okamoto[b]	I	II	NC[l]	III	IV						
Mori[c]	I	II	NC	III	IV	NC	V	VI			
Cha[d]	GI	GII	NC	GIII	GIII	NC	GIV	GIV	NC	GV	
Nakao[e]	Pt	K1	NC	K2a	K2b	NC	K3	K3			
Bukh[f]	I/1a	II/1b	NC	III/2a	IV/2b	NC	(V)/3a	NC	4a	5a	6a
Prototype	HCV-1[g]	HCV-J[j]		HC-J6[b]	HC-J6[b]		E-b1		Z6[f]	SA3[f]	HK1
	HCV-H[f]	HCV-BK[k]					Ta[c]	Tb[c]	EG-1[b]	SA4[f]	HK2
		HCV-J1[h]					Br36		to	SA1[f]	HK3
		HCV-J4[i]					Br33		EG-33[b]	SA3[f]	HK4
		HC-JK1					HD10		BU79	SA7[f]	
									BU74	SA11[f]	
									GB80	PC	
									GB116		
									GB549		
									GB809		

[a]Simmonds et al. [1993b].
[b]Okamoto et al. [1992b].
[c]Mori et al. [1992].
[d]Cha et al. [1992].
[e]Nakao et al. [1991].
[f]Bukh et al. [1992].
[g]Choo et al. [1991].
[h]Okamoto et al. [1990].
[i]Okamoto et al. [1991].
[j]Kato et al. [1990].
[k]Takamizawa et al. [1991].
[l]NC, not classified.

not yet been reported. The major aim of this study is to investigate the distribution HCV genotypes in Korea among disease groups: blood donors, patients with HCC, patients with chronic hepatitis or liver cirrhosis, and patients on maintenance hemodialysis.

MATERIALS AND METHODS

Patients and Plasma

Sera from 30 blood donors, 30 patients with HCC, 33 patients with chronic hepatitis, 15 patients with liver cirrhosis, and 30 patients on maintenance hemodialysis who were anti-HCV antibody (anti-HCV) and HCV PCR-positive, were frozen immediately at −80°C until analysis.

Anti-HCV Antibody Assay

Enzyme immunoassay for determination of anti-HCV was used with a second-generation kit (HCV EIA 2nd generation, Abbott Laboratories, North Chicago, IL) using antigens of 5-1-1, C33c, and C22c.

HCV PCR With Amplicor HCV Kit

Combined reverse transcription polymerase chain reaction (RT-PCR) with Amplicor™ HCV PCR kit (Roche, Branchburg, NJ, U.S.A.) was done according to the instructions of the kit. In brief, HCV RNA extraction was performed using a lysis buffer (5.7 M guanidium thiocyanate, 49.5 mM Tris HCl [pH 7.5], 100 mM beta-mercaptoethanol, and 1.25 μg of poly(rA) per ml) and the RNA was then precipitated with isopropanol using the method of Young et al. [1995]. Reverse transcription and amplification was carried out for 40 cycles (2 cycles at 95°C for 15 sec and at 60°C for 20 sec: 39 cycles at 90°C for 15 sec and at 60°C for 20 sec) in a Geneamp PCR system 9600 thermal cycler (Perkin Elmer, Norwalk CT) using rTth polymerase. The PCR product was confirmed by DNA enzyme immunoassay.

HCV Subtyping by PCR

Typing of HCV was done by method of Okamoto et al. [1992a]. Based on the comparison of a portion of putative core gene, universal primers and a mixture of four type-specific primers were used.

INNO LiPA Test

HCV cDNA was primed with no. 186 primer and amplified with outer HCV primer for 40 cycles (Table II). The reaction cycle included denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. One-tenth of the PCR product was subjected to nested PCR with inner primers in the same condition as the first PCR. The typing strip tests were done with 10 μl of nested PCR product by the reverse hybridization method with INNO-LiPA HCV (Innogenetics N.V., Zwinaarde, Belgium).

RESULTS

Among the 138 HCV PCR-positive patients, type 1b was the prevailing type (98 out of 138, 71%), followed by type 2a (33 out of 138, 23.9%), type 1a (3 out of 138, 2.1%), type 2b (2 out of 138, 1.5%) and unclassified (2 out of 138, 1.5%) in Korea (Table III). In 30 blood donors, HCV genotype 1b was most dominant (24 out of 30, 80%), followed by genotype 2a (2 out of 30, 13.3%), and 2b (2 out of 30, 6.7%). For 30 HCC cases, the distribution of four HCV genotypes was slightly different from that of blood donors. HCV genotype 1b was less frequent (18 out of 30, 60%) compared to blood donors, followed by

TABLE II. Primers and Probes Used in HCV RT-PCR for INNO-LIPA Test

Name	Position	Polarity	Sequence 5′ to 3′
No. 186	391	−	ATGTACCCCATGAGGTCGGC
Outer P	−299	+	CCCTGTGAGGAACTWCTGTCTTCACGC
Outer P	−1	−	GGTGCACGGTCTACGAGACCT
Inner P	−264	+	TCTAGCCATGGCGTTAGTRYGAGTGT
Inner P	−29	−	CACTCGCAAGCACCCTATCAGGCAGT
1	−170	+	AATTGCCAGGACGACC
1	−117	−	TCTCCAGGCATTGAGC
1b	−103	+	CCGCGAGACTGCTAGC
2	−126	−	ATAGAGTGGGTTTATC
2	−83	+	TAGCGTTGGGTTGCGA
2a	−165	+	CCGGGAGACTGGGTC
2a	−136	+	ACCCACTCTATGCCCG
2b	−165	+	CCGGAAAGACTGGGTC
2b	−136	+	ACCCACTCTATGTCCG
3	−170	+	AATCGCTGGGGTGACC
3	−117	−	TTTCTGGGTATTGAGC
3	−103	+	CCGCGAGATCACTAGC
3	−146	+	TCTTGGAGCAACCCGC
4	−170	+	AATYGCCGGGATGACC
4	−117	−	TTTCCGGGCATTGAGC

TABLE III. Distribution of HCV Genotypes in Korea

		Genotype sample				
Disease	Number	1a	1b	2a	2b	etc.
Blood donors	30	0	24	4	2	
		0%	80.0%	13.3%	6.7%	
Chronic hepatitis	33	2	21	10	0	
		6.1%	63.6%	30.3%	0%	
Liver cirrhosis	15	1	9	5	0	
		6.7%	60.0%	33.3%	0%	
HCC	30	0	18	10	0	2
		0%	60.0%	33.3%	0%	6.7% (unclassified)
Hemodialysis	30	0	26	4	0	
		0%	86.7%	13.3%	0%	
Total	138	3	98	33	2	2
	100%	2.1%	71.0%	23.9%	1.5%	1.5% (unclassified)

genotype 2a (10 out of 30, 33.3%), and unclassified (2 out of 30, 6.7%). For 33 cases of chronic hepatitis, HCV genotype 1b was also dominant (21 of 33, 63.6%), followed by genotype 2a (10 out of 33, 30.3%), and 1a (2 out of 33, 6.1%). In 15 patients with liver cirrhosis, HCV genotype 1b was also dominant (9 out of 15, 60%), followed by genotype 2a (5 out of 15, 33.3%), and 1a (1 out of 15, 6.7%). For 30 patients on maintenance hemodialysis, HCV genotype 1b was dominant (26 out of 30, 86.7%), followed by genotype 2a (4 out of 30, 13.3%).

The HCV typing was carried out using Okamoto's typing method and multiple bands of PCR product. Of 138 cases, analysis of 40 cases (28.9%) resulted in multiple bands (two or three bands), which had been frequently mistaken as mixed infection. For these 40 cases, INNO-LiPA typing was carried out. Of 40 cases, clear decision on the HCV type was made in 38 cases, and two cases showed only a positive band for the amplification control, with no other bands. It is concluded that these two cases, which were also HCV PCR-positive, using the Amplicor™ HCV PCR kit, would be unclassified types. We plan to clone and sequence the PCR products of these two cases.

TABLE IV. Comparison of HCV Genotypes Between Korea and Japan*

	Genotype (%)					
Disease	1a	1b	2a	2b	etc.	
Blood donors	4	82	10	4	0	Japan
	0	80	13	7		Korea
Chronic hepatitis	0	59	26	0	1	Japan
	6	64	30	0		Korea
Liver cirrhosis	8	63	21	8	0	Japan
	7	60	33	0		Korea
HCC	11	47	18	18	1	Japan
	0	60	33	0	7	Korea

*The distribution of HCV genotypes in Japan is based on Okamoto's et al. study [1992a].

Some differences were noted in the distribution of HCV between blood donors and patients with liver diseases (Tables IV and V). Although type 1b was the most prevalent type in both donors and patients with liver disease, it was found more often in donors than in the patients (P value = 0.08).

TABLE V. Incidence of HCV Genotype 1b in Blood Donors and Patients With Chronic Liver Disease

Type/ Disease	Type 1b* No. (%)	Type 2a No. (%)
Blood donor (n = 30)	24 (80.0%)	4 (13.3%)
Liver disease[a] (n = 78)	48 (61.5%)	25 (32.1%)

[a]Liver disease: patients with chronic hepatitis, or liver cirrhosis, or hepatocellular carcinoma.
*Chi-square test, P value = 0.08, marginally significant increase of incidence of type 1b among blood donors.

DISCUSSION

HCV is a RNA virus with higher variation in genomic sequence. In general, several genotypes are distributed around the prototype resident in a single area. Different HCV genotypes have been reported in American and Japanese populations [Kato et al., 1991; Inchauspe et al., 1991; Choo et al., 1991]. In Japan, HCV type 1b is the most prevalent type both in apparently healthy blood donors (82%) and patients with non-A, non-B (NANB) liver disease (60%) [Okamoto et al., 1992a]. In contrast, the HCV isolated from Americans were predominantly type 1a [Ulrich et al, 1990], as were those from the British [Simmonds et al., 1990]. In northern Italy, HCV type 1b is most prevalent (54.2%) and type 1a accounts for only 13% of the infections [Ravaggi et al., 1994]. Although classification of HCV subtypes has not yet been standardized, the classification of Okamoto et al. [1992b, 1993] is widely used, based on the heterogeneity of the capsid or NS-5 region of HCV. Recently, Simmonds et al. [1993a] classified HCV into six major genotypes, based on the nucleotide sequence of E1 or NS-5 region of HCV. There is some confusion about the nomenclature of HCV subtypes. A comparison of various classification is described in Table I. Several methods of HCV subtyping were introduced: core serotype [Mondelli et al., 1994], NS4 serotyping [Mizokami et al., 1994], Line probe assay [Vadon-Doorn et al., 1994], restriction fragment length polymorphism (RFLP) [McOmish et al., 1994; Nakao et al., 1991], and PCR typing with core region [Okamoto, 1992].

Variation in a viral genome changes the kinetics of replication, cell-specific tropism, and response to neutralizing antibodies. Thus, it is probable that different genotypes of HCV might result in different diseases, but there is much controversy about the role of HCV genotypes in diseases. Pozzato et al. [1994] reported that different genotypes of hepatitis C virus were associated with differing severity of chronic liver disease. According to the above report, patients infected with genotype 1b were more likely to develop liver cirrhosis. McOmish et al. [1993] also reported that infection with HCV type 2a might be associated with more severe liver disease, while Yamata et al. [1994] reported that HCV genotypes were not relevant to the likelihood of the development of serious liver disease. Variable responses to interferon therapy have been reported [Mita et al., 1994; Sakai et al., 1994]. Differences in serologic reactivity of anti-HCV antibody among HCV genotypes have been recently reported [Alonso et al., 1994: McOmish et al., 1993: Mondelli et al., 1994: Nagayama et al., 1993].

Through an international collaborative survey in Europe and eastern Asia, McOmish et al [1994] reported the distribution of HCV genotypes in blood donors. Types 1, 2, and 3 accounted for almost all infections in donors from Scotland, Finland, Netherlands, and Australia. Types 2 and 3 were not found in Hungary, where all but one of the donors were infected with type 1. Donors from Japan and Taiwan were infected only with types 1 or 2, while types 1, 2, and 6 were found in those from Hong Kong. HCV infection among Egyptians was almost always with type 4. In this study, HCV genotypes found in Korea were 80% for type 1b, 14% for type 2a, and 6% for type 2b. A novel HCV type [Mori et al., 1992; Bukh et al., 1992; Simmonds et al., 1993b], designated type 6, was not found in blood donors in Korea.

High incidence of HCV infection among patients on maintenance hemodialysis is reported, varying from 5% to 30% [Dussol et al., 1995; Gracia-Valdecasas et al., 1994]. Hadiwandowo et al. [1994] reported the distribution of HCV genotypes in patients on maintenance hemodialysis in China. In their study, of 44 samples from patients on maintenance hemodialysis, 39 (89%) were genotype 1a and only one (2%) was genotype 1b, which was strikingly different from the genotype distribution of chronic liver disease in China. Among HCV RNA-positive samples from 48 patients with chronic liver disease in China, 23 (48%) were of genotype 1b, 17 (35%) of genotype 2a and one (2%) of genotype 3a. According to the study of Chou et al. [1993] among patients on maintenance hemodialysis in China, 18.2% were of type II (type 1b) and 81.8% were of type III (type 2a), using Cha's classification. In this study, HCV genotype distribution of patients on maintenance hemodialysis in Korea was found to be markedly different from that of China, revealing that type 1b (86%) was dominant in Korea.

Okamoto et al. [1992a] reported that there was a significant difference in the distribution of HCV genotypes between donors and patients. Although type 1b was the most prevalent type in both donors and patients with NANB liver disease in Japan, it was found more frequently in donors (82%) than in patients (60%). In that study, the prevalence of type 2a was lower in donors (10%) than in patients (23%), and the prevalence of type 1b was the lowest (47%) in patients with HCC. Our results were similar to those of Okamoto's et al. In Korea, the prevalence of type 1b was marginally different between blood donors and patients with HCC (P value = 0.06).

In conclusion, among 138 anti-HCV antibody-positive patients, type 1b was the prevailing type (98 out of 138, 71%), followed by type 2a (33 out of 138, 23.9%), type 1a (3 out of 138, 2.1%), type 2b (2 out of 138, 1.5%), and unclassified (2 out of 138, 1.5%) in Korea. No significant difference in HCV genotype distribution was found among blood donors, patients with HCC, patients with

chronic hepatitis or liver cirrhosis, and patients on maintenance hemodialysis.

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